

( i i ) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GCGCAAGCTT TTTTTTTTTT AA

2 2

( 2 ) INFORMATION FOR SEQ ID NO: 29:

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 22
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

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We claim:

1. Method for detecting different stages of cell development or detecting differences in gene expression comprising the steps of:

- a) contacting mRNA from each of said cell populations in separate reaction vessels with a first oligonucleotide primer, wherein said first oligonucleotide primer has a hybridizing sequence sufficiently complementary to a region of said mRNA to hybridize therewith,
- b) extending said first oligonucleotide primer in an extension reaction using the mRNA as a template to give a first DNA primer extension product complementary to the mRNA,
- c) contacting said first DNA primer extension product with a second oligonucleotide primer, wherein said second oligonucleotide primer has a hybridizing sequence sufficiently complementary to said first DNA primer extension product to hybridize therewith,
- d) extending said second oligonucleotide primer in an extension reaction using the first DNA primer extension product as a template to give a second DNA primer extension product complementary to the first DNA primer extension product,
- e) amplifying said first and second DNA primer extension products in a polymerase chain reaction comprising cycles of primer annealing, extension and denaturation, at an annealing temperature of between 35° and 45° for at least two and not more than four cycles, then
- f) amplifying said first and second DNA primer extension products at an annealing temperature of between 55° and 70° for at least 16 cycles, to provide amplified gene sequences
- g) separating said amplified gens sequences by size and/or charge; and
- h) comparing amplified gens sequences separated in step (g) to detect an amplified gene sequence from one of said cell populations that is present at a different level in the other of said cell populations;

wherein said first and second oligonucleotide primers comprise at least 21 nucleotides.

2. The method of claim 1 wherein a mixture of two or more first primers is used.

3. The method of claim 1 wherein a mixture of two or more second primers is used.

4. The method of claim 1 wherein a mixture of two or more first primers and a mixture of two or more second primers are used.

5. The method of claim 1 wherein said first and second oligonucleotide primers consist of from 21 to 50 oligonucleotides.

6. The method of claim 1 wherein said first oligonucleotide primer contains a restriction site.

7. The method of claim 1 wherein said second oligonucleotide primer contains a restriction site.

8. The method of claim 4 wherein said first oligonucleotide primer hybridizes to a region of mRNA comprising a portion of a 3' polyadenosine tail of said mRNA and at least one nucleotide 5' to said 3' polyadenosine tail.

9. The method of claim 4 wherein said first primers are selected from the group consisting of

5'-GCG CAA GCT TTT TTT TTT TTC T-3' (SEQ ID NO. 19);

5'-GCG CAA GCT TTT TTT TTT TTC C-3' (SEQ ID NO. 20);

5'-GCG CAA GCT TTT TTT TTT TTC G-3' (SEQ ID NO. 21);

5'-GCG CAA GCT TTT TTT TTT TTG T-3' (SEQ ID NO. 22);

5'-GCG CAA GCT TTT TTT TTT TTG G-3' (SEQ ID NO. 23);

5'-GCG CAA GCT TTT TTT TTT TTG A-3' (SEQ ID NO. 24);

5'-GCG CAA GCT TTT TTT TTT TTA T-3' (SEQ ID NO. 25);

5'-GCG CAA GCT TTT TTT TTT TTA C-3' (SEQ ID NO. 26);

5'-GCG CAA GCT TTT TTT TTT TTA G-3' (SEQ ID NO. 27);